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Analysis of oxidized biodiesel by ^{1}H-NMR and effect of contact area with air*

Biodiesel is continuously gaining attention and significance as an alternative diesel fuel. An important issue facing biodiesel is fuel stability upon exposure to air due to its content of unsaturated fatty acids. Numerous factors influence the oxidative stability of biodiesel, and several methods for its assessment have been developed. In the present work, a defined amount of biodiesel (methyl soyate) was heated in open beakers, with the only difference being the size of the beaker, *i.e.* the surface area of the biodiesel exposed to air. Biodiesel oxidized in this fashion was analyzed by ^{1}H-NMR, kinematic viscosity and acid value. Acid values and kinematic viscosity increased with time and surface area. A previously developed ^{1}H-NMR procedure was used to evaluate the unsaturation and "residual" fatty acid composition. The amounts of saturated fatty acids determined by this method increased, with monounsaturated and diunsaturated species increasing and then decreasing with time. After "flash" (3 h, 165 °C) oxidation, NMR shows the greatest effect on saturates and compounds with two double bonds, the former increasing and the latter decreasing. The double bond originally located at Δ15 in 18:3 is largely retained, showing that other double bond positions in 18:3 are initially affected by oxidation. The methyl ester signal decreases, coinciding with the increase in acid value. An increasingly strong absorption was observed in the UV-VIS spectra. Increasing surface area accelerated oxidation and affected fatty acid composition.

Keywords: Acid value, biodiesel, fatty acid methyl esters, kinematic viscosity, nuclear magnetic resonance, oxidation.

1 Introduction

Production and use of biodiesel [1, 2], an alternative diesel fuel obtained by transesterification of vegetable oils or other materials largely consisting of triacylglycerols such as animal fats or used frying oils, with monohydric alcohols to give the corresponding mono-alkyl esters has increased significantly in recent years. Biodiesel is still confronted with some technical challenges such as reducing nitrogen oxides (NO_x) exhaust emissions as well as improving oxidative stability and cold flow properties. Advantages of biodiesel compared to petrodiesel include reduction of most exhaust emissions, improved biodegradability, inherent lubricity, higher flash point, and domestic origin. Virtually no changes in the fuel distribution infrastructure are required when using biodiesel.

The increasing use of biodiesel has led to a corresponding significance of the issue of fuel quality, which includes the exposure of biodiesel to air (oxygen). Numerous studies [3–24] have reported the influence of factors such as the presence of light, elevated temperature, metal, the nature of the container, and other extraneous materials on the fuel quality of biodiesel. Additionally, commercial biodiesel can be exposed to elevated temperatures, especially during storage in larger tanks in warm weather conditions. Generally, the effect of these factors is that they catalyze oxidation. Oxidation is a complex process [25], ultimately leading to a variety of species including shorter-chain fatty acids and aldehydes, but also to higher-molecular-weight species through oxidative polymerization.

In the previous studies, numerous methods for assessing the oxidation status of biodiesel have been investigated, including acid value, density, and kinematic viscosity. The peroxide value may not be suitable [11, 13, 15] because, after an initial increase, it decreases due to secondary oxidation reactions, although the decrease likely affects only samples oxidized beyond what may normally be

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expected. Thus, there is the possibility of the fuel having undergone relatively extensive oxidation but displaying an acceptable peroxide value. The peroxide value is also not included in biodiesel standards. Acid value and kinematic viscosity, however, are two facile methods for rapid assessment of biodiesel fuel quality as they continuously increase with deteriorating fuel quality [11].

The surface area between a liquid and a gas is a significant factor influencing interphase transport phenomena and reactions. Accordingly, the contact area between the biodiesel was varied by changing the size of the open beaker in which it was heated, but not the amount of the fuel. In order to analyze the biodiesel oxidized in this fashion, the resulting material was analyzed by proton nuclear magnetic resonance (^{1}H-NMR), which is of specific interest because the fatty acid profile of biodiesel (or a vegetable oil of fat) can be determined easily by this method [26] and changes to the peaks generated by the presence of the double bonds are affected by oxidation. Thus, the kinds of fatty compounds in oxidized biodiesel should be quantifiable by NMR, especially in light of the fact that few details are known about the fatty acid composition of oxidized biodiesel. NMR has been applied extensively to lipid oxidation [27], with some recent work assessing the formation of species such as aldehydes and shorter-chain acids [28]; however, to the best of our knowledge, a method for calculating the fatty acid profile was not applied. The results of the NMR determination are compared to analyses by acid value, kinematic viscosity, gel permeation chromatography (GPC) and electronic (UV-VIS) spectroscopy.

2 Materials and methods

Commercial methyl soyate (biodiesel) was obtained from Ag Environmental Products (trade name SoyGold; Lenexa, KS, USA). The biodiesel (50 mL in all experiments) was heated on a hot plate in a hood in open glass beakers of different sizes without stirring, as slight variations in stir speed may also affect oxidation. Tab. 1 reports analytical data on the methyl soyate used as biodiesel, including the fatty acid profile by gas chromatography (GC) carried out in comparison to ^{1}H-NMR. A separate silicone oil bath with contact thermometer was placed on the hot plate next to the sample and used for regulating the temperature of the hot plate. Thus, the surface area was the only variable in these experiments. Reactions were carried out in 100-, 150-, and 250-mL beakers. The volume-to-surface area ratios of these beakers were 2.8, 2.2, and 1.7 mL/cm^3, respectively. The reaction conditions were 3 h at 165 °C ("flash" oxidation) as well as 48 h and 168 h at 80 °C.

Tab. 1. Fatty acid profiles by GC and ^{1}H-NMR, kinematic viscosity (40 °C), acid value and OSI time (110 °C) of the biodiesel fuel used in this work[§].

Fatty acid composition		
	GC	**NMR**
C16:0	10.96	
C18:0	4.09	15.50[§]
C18:1[$]	23.61	26.62
C18:2	52.84	48.76
C18:3	7.68	9.12
Kinematic viscosity: 4.1 mm^2/s		
Acid value: 0.26		
OSI (110 °C): 3.12 h		

[§] Saturates not distinguished by ^{1}H-NMR.
[$] 0.76% 18:1 Δ11.
[#] Oil stability index (OSI), AOCS method Cd12b-92 [29]. Mean of three runs, standard deviation = 0.08 h.

NMR spectra were obtained on a Bruker (Billerica, MA, USA) Avance 500 spectrometer operating at 500 MHz with $CDCl_3$ as solvent. GC analyses were performed on a Hewlett-Packard (Palo, Alto, CA, USA) Series II gas chromatograph equipped with a flame ionization detector and a Supelco (Bellefonte, PA, USA) SP-2560 capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness). UV-VIS spectra were obtained with hexane as solvent using a Perkin-Elmer (Norwalk, CT, USA) Lambda 5 spectrophotometer. Kinematic viscosity values were determined with Cannon-Fenske viscometers (Cannon Instrument Co., State College, PA, USA) at 40 °C following the standard method ASTM D445 [30]. All viscosity data reported here are means of triplicate determinations. Acid values were obtained using AOCS (American Oil Chemists' Society) method Cd 3d-63 [31]. Gel permeation chromatography was carried out an a Polymer Laboratories (Amherst, MA, USA) PL-GPC 120 instrument with refractive index detector and a PL gel 3 μm MIXED-E 300 × 7.5 mm column (Polymer Laboratories) using tetrahydrofuran as solvent.

3 Results and discussion

^{1}H-NMR was chosen as the primary analytical method since oxidized biodiesel samples may not be amenable to GC-based analyses, due to the formation of higher-molecular-weight species. Such species have been analyzed in heated biodiesel [14]. However, ^{1}H-NMR can be used for fatty acid profile determination [26] and therefore it was of interest to apply this method to oxidized biodiesel and to compare these data to other easily conducted

methods for assessing fuel quality, such as kinematic viscosity and acid value. Besides these analytical aspects, the influence of varying the surface area towards air of a constant volume of biodiesel was assessed.

When applying ^{1}H-NMR, some limitations of the method must be kept in mind, affecting mainly 18:2 species and mainly based on accuracy of integration. The data in Tab. 1 show slight deviations of the fatty acid composition when using ^{1}H-NMR, which was also discussed previously [26]. Therefore, a corresponding deviation of the results obtained here from those that would be theoretically obtainable by chromatography-based methods must be assumed. This observation, however, does not affect the background of the phenomenon and resulting visible trends discussed here.

The fatty acid profile of a vegetable oil with the common species 18:1, 18:2, 18:3 as well as saturates can be calculated using the following equations utilizing the integration values of certain peaks [26]. Thus, the amount of 18:3 ($A_{18:3}$) is given by:

$$A_{18:3} = I_{exper,methyl,18:3}/(I_{exper,methyl,18:3} + I_{exper,methyl,rest}) \quad (1)$$

in which $I_{exper,methyl,18:3}$ is the integration value of the terminal CH_3 protons of linolenic acid and $I_{exper,methyl,rest}$ is the integration value of the terminal CH_3 protons of all other fatty acids in the sample, since the double bond at C15 causes a downfield shift of the terminal methyl protons.

With $A_{18:3}$ determined in this fashion, the amount of 18:2 fatty acids ($A_{18:2}$) can be determined by the equation [26]:

$$A_{18:2} = 0.5\ (I_{exper,bisallylic} - 4A_{18:3}) \quad (2)$$

in which $I_{exper,bisallylic}$ is the experimentally determined integration value for the bis-allylic protons. The amounts of diunsaturated fatty acid moieties are slightly underestimated by this method [26, 32].

The amount of monounsaturated fatty acids ($A_{18:1}$) is given by [26]

$$A_{18:1} = (I_{exper,allylic}/4) - A_{18:2} - A_{18:3} \quad (3)$$

in which $I_{exper,allylic}$ is the experimentally determined integration value for the allylic protons. The amount of saturated fatty acids can then be determined by adding the amount of unsaturated species (in%) and subtracting from 100%.

For the analysis in this work, the protons of the carbon α to the methyl ester functionality were used as reference with an integration value of 2. Applying the signals of these protons is especially useful because they are present in all acids and esters. Since shorter-chain acids are formed during oxidation, it is more appropriate in the further discussion to refer to the fatty acids in oxidized biodiesel as saturated, monounsaturated, diunsaturated and residual ω-3 compounds (see discussion below for this term), instead of including a reference to C_{16} or C_{18} compounds. This aspect, however, does not affect quantification by the equations given here.

Tab. 2 gives the kinematic viscosity and acid values of the oxidized samples. The fatty acid composition of the oxidized samples as calculated from Eqs. (1)–(3) is contained in Tab. 3. Fig. 1 depicts the ^{1}H-NMR spectrum of commercial methyl soyate, Fig. 2 shows methyl soyate oxidized at 165 °C for 3 h and Fig. 3 contains the ^{1}H-NMR spectrum of a sample oxidized at 80 °C for 168 h. Differences in the NMR spectra of the oxidized samples are clearly visible and the results of their evaluation are discussed below.

The fatty acid composition of the oxidized biodiesel determined by the present NMR method shows an enrichment of monounsaturated and saturated fatty acids, with an accompanying decrease of diunsaturated species. The saturated species may arise either through chain cleavage, leading to shorter-chain acids, or saturation of unsaturated species. Interestingly, quantitation by the present method implies no or little change of the amount of triunsaturated (18:3) fatty acid, but this cannot be equated with actually existing 18:3 as the following considerations show. While fatty acids with methylene-interrupted double bonds such as linolenic acid (and its esters) are especially susceptible to oxidation, the original Δ15 double bond in 18:3 is largely retained, showing that other double bond positions in 18:3 are preferentially affected by the oxidation process. In other words, likely due to its position close to one end of the fatty acid chain, the Δ15 double bond is less susceptible to oxidation. Thus, the species formed from 18:3 likely contain a higher percentage of ω-3 mono- and diunsaturated fatty acid chains than might be expected. Therefore, the species showing ω-3 unsaturation are termed "residual ω-3" compounds, although they likely are 18:1 or 18:2 compounds.

Tab. 2. Kinematic viscosities (40 °C) and acid values of oxidized biodiesel. A volume of 50 mL biodiesel was used for each oxidation test. Standard deviations are given in parentheses.

Oxidation conditions	Kinematic viscosity [mm^2/s]	Acid value
80 °C; 168 h; 100-mL beaker	6.37 (1.73)	2.35 (1.85)
80 °C; 168 h; 150-mL beaker	17.53 (1.71)	10.31 (1.41)
80 °C; 168 h; 250-mL beaker	22.66 (1.37)	13.09 (3.35)
165 °C; 3 h; 100-mL beaker	4.84 (0.11)	0.65 (0.03)
165 °C; 3 h; 150-mL beaker	5.21 (0.05)	0.83 (0.06)
165 °C; 3 h; 250-mL beaker	6.05 (0.31)	0.94 (0.09)

Tab. 3. NMR evaluation of oxidized biodiesel applying Eqs. (1)–(3). Standard deviations are given in parentheses.

Oxidation conditions	Number of experiments	Fatty acid composition [%]			
		Saturates	Monounsaturates	Diunsaturates	Residual ω-3[§]
80 °C; 168 h; 100-mL beaker	3	31.92 (9.13)	29.46 (0.95)	31.24 (9.83)	7.38 (0.56)
80 °C; 168 h; 150-mL beaker	3	61.17 (0.95)	25.39 (3.51)	8.38 (5.46)	5.06 (1.46)
80 °C; 168 h; 250-mL beaker	3	67.58 (2.24)	24.27 (1.22)	4.30 (1.44)	3.85 (0.09)
165 °C; 3 h; 100-mL beaker	3	23.58 (1.23)	29.94 (0.36)	38.28 (0.73)	8.19 (0.15)
165 °C; 3 h; 150-mL beaker	4	26.07 (0.59)	30.47 (0.47)	35.44 (0.22)	8.03 (0.30)
165 °C; 3 h; 250-mL beaker	5	29.15 (1.57)	31.96 (0.85)	30.77 (1.21)	8.11 (0.30)

[§] This column designation refers to the fact that the corresponding ^{1}H-NMR signal originally present in 18:3 is preserved and does not indicate that the compounds are triunsaturates. See also discussion in the text.

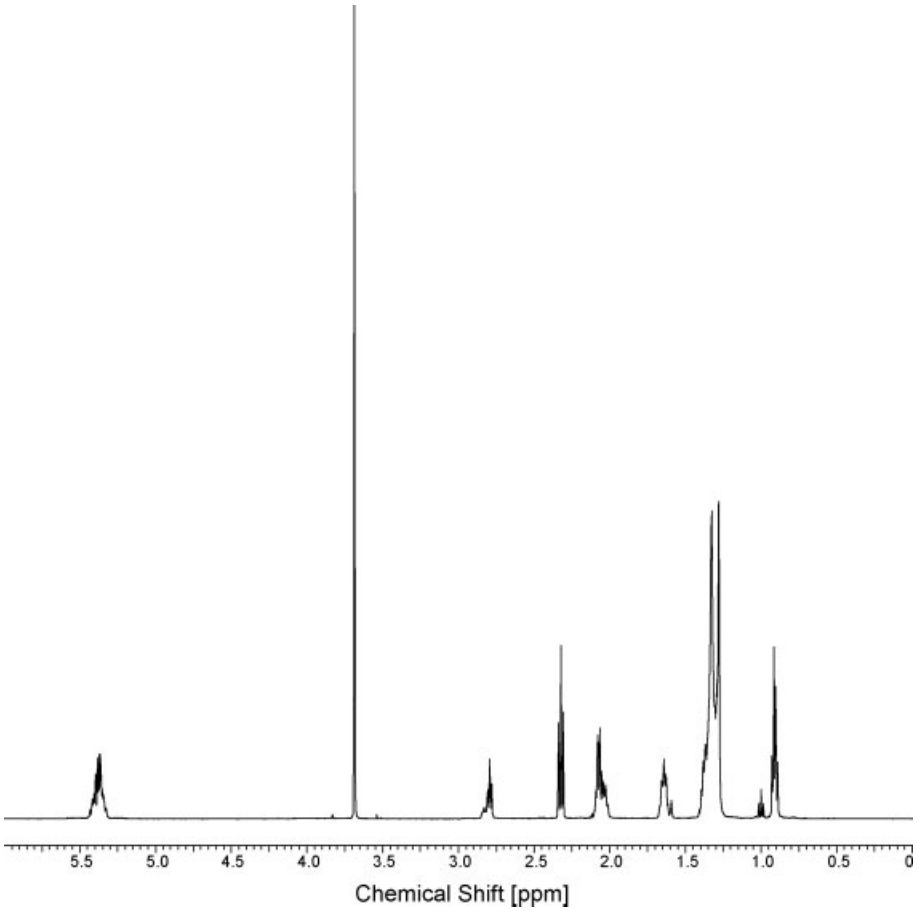

Fig. 1. ^{1}H-NMR spectrum of an unoxidized methyl soyate (biodiesel).

The monounsaturates in all samples either increased or were close to the 18:1 component of the methyl soyate starting material. The data in Tab. 3 imply an initial increase in monounsaturates, followed by a decrease as saturates increase, depending on oxidation time and severity.

The singlet peak caused by the methyl ester moiety decreases during the reaction, which can be explained by formation of free fatty acids (FFA). The remaining amount of methyl ester $A_{\text{me ester}}$ is given by

$$A_{\text{me ester}} = I_{\text{exper,me ester}}/3 \quad (4)$$

in which $I_{\text{exper,me ester}}$ is the experimentally determined value for the singlet peak caused by the methyl ester protons. The greatest decreases of the peak of the methyl ester moiety were observed for the most heavily oxidized

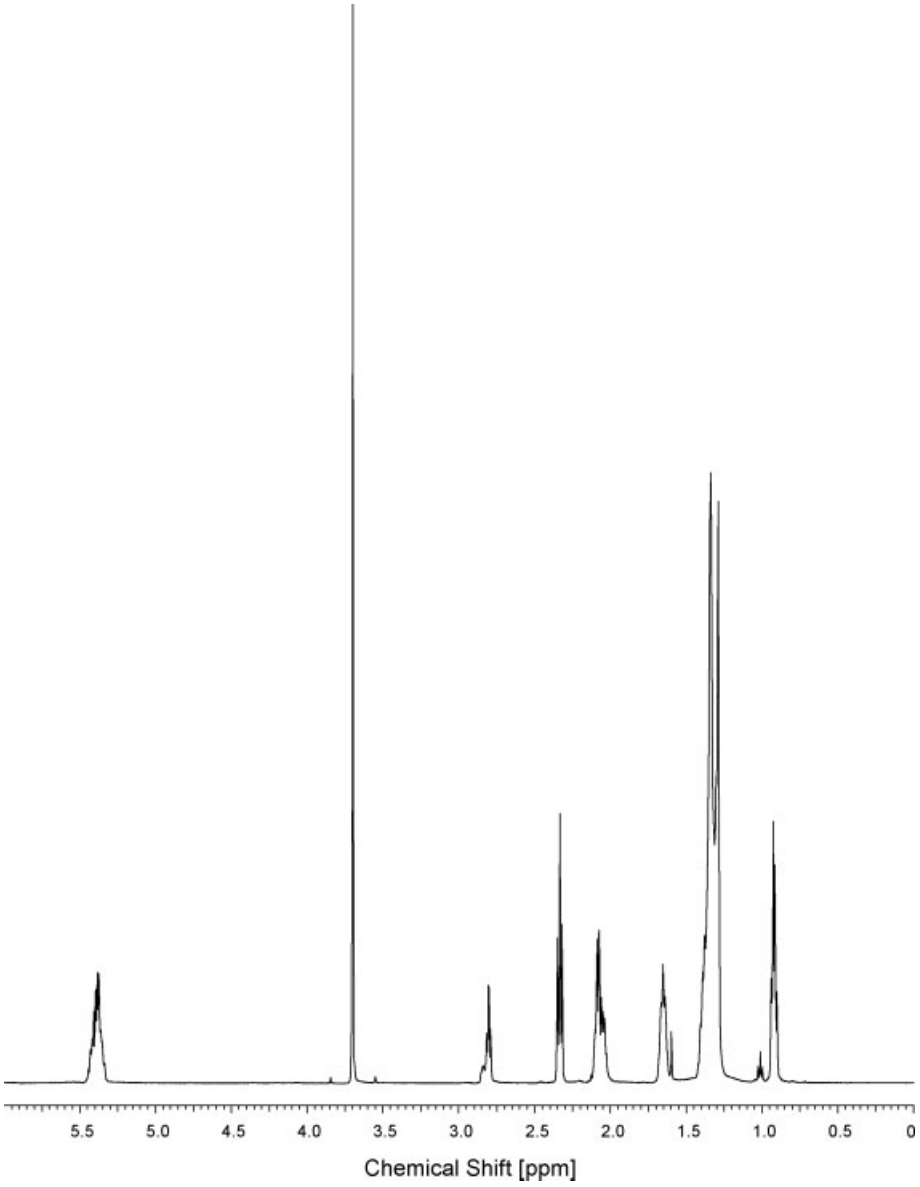

Fig. 2. ^{1}H-NMR spectrum of 50 mL biodiesel oxidized in a 100 mL beaker at 165 °C for 3 h.

species (250-mL beaker, 80 °C for 168 h), for which values of $A_{\text{me ester}}$ of about 90% were observed. This coincides with these samples also possessing the highest acid values (Tab. 2). For the sake of comparison, reactions conducted at 80 °C for 48 h (not contained in Tab. 2) showed less increased acid and kinematic viscosity values. For example, the acid and kinematic viscosity values of biodiesel oxidized in the 250-mL beaker were 3.75 and 8.06 mm^2/s, respectively.

The NMR evaluation showing increasing saturated and monounsaturated species coincides well with the increasing kinematic viscosity and acid values. Saturated fatty compounds possess higher viscosity than their unsaturated counterparts; however, this effect may be mitigated by the formation of shorter-chain acids. However, FFA also exhibit greater viscosity than methyl esters. For example, the kinematic viscosity of oleic acid is 19.91 mm^2/s and that of methyl oleate is 4.51 mm^2/s [33]. Not only the formation of FFA and more saturated species will increase viscosity, but also that of higher-molecular-weight species. GPC investigations of a few samples generated indicated a slight increase of higher-molecular-weight species to the dimer or, less, the trimer range, and may warrant further investigation beyond the scope of the present work. No conjugation of double bonds in the chains, which give very specific peaks in ^{1}H-NMR [34], was observed. The formation of dienes other than 1,3- or

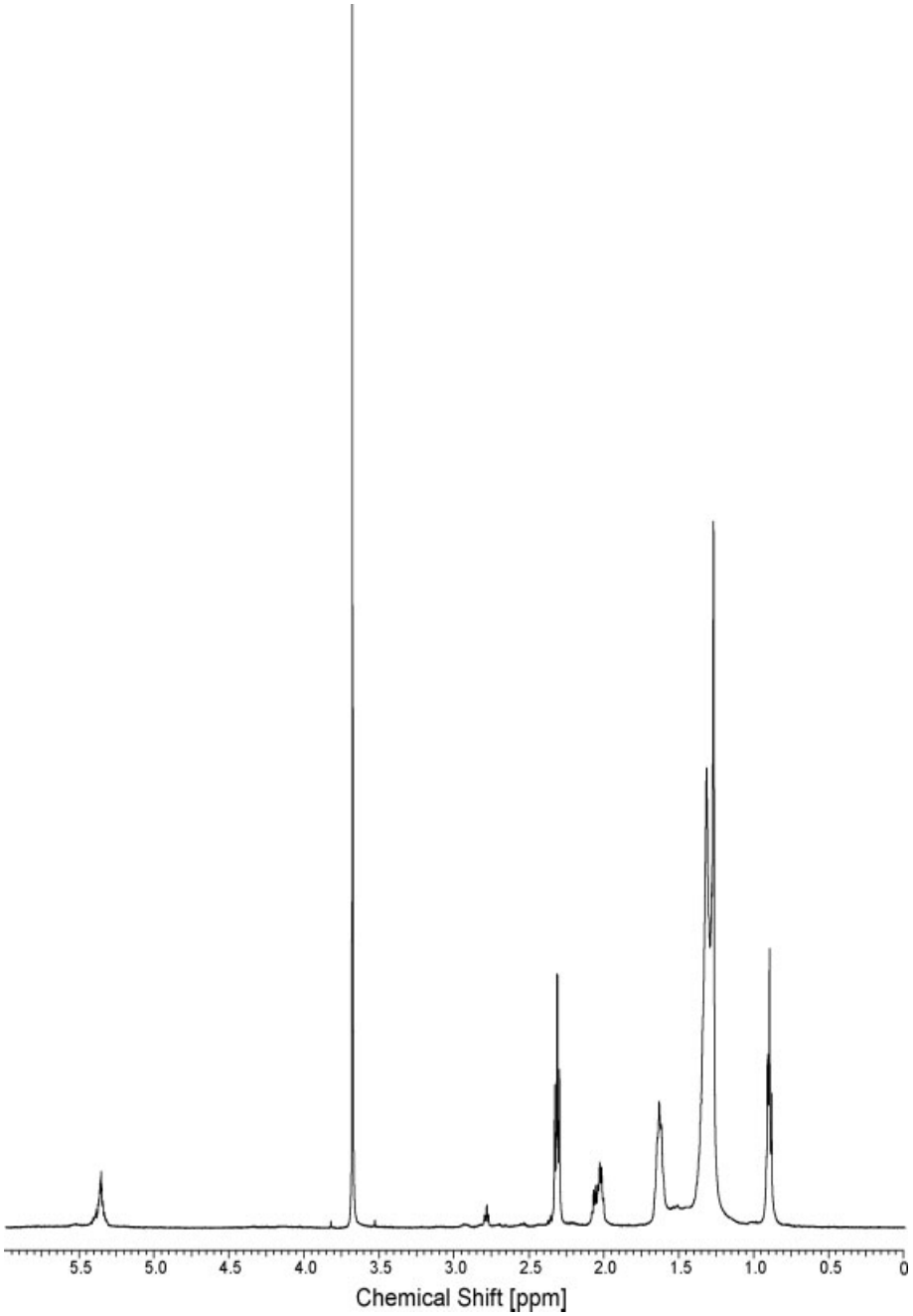

Fig. 3. ^{1}H-NMR spectrum of 50 mL biodiesel oxidized in a 250 mL beaker for 168 h at 80 °C.

1,4-dienes as analyzed here cannot be fully excluded and their evaluation, due to the separation of the double bonds, would be included in the 18:1 species.

UV-VIS spectra of the oxidized samples were obtained in hexane and compared to the original biodiesel fuel sample. Originally, the methyl soyate shows a maximum at about 250 nm. With increasing oxidation, the samples show a strong absorption below 300 nm overriding other absorption maxima, the onset of which increases to about 350 nm with increasing oxidation. These results largely coincide with literature results [35]. However, this method may warrant further investigation as procedure for determining the oxidation status of biodiesel since UV-VIS spectra are rapidly and easily acquired and instrumentation is moderate in price.

As the data in Tabs. 2 and 3 show, the surface area between the biodiesel fuel and air plays a significant role in oxidation. The differences become more pronounced

with increasing oxidation time as the comparison of the reactions conducted under "flash" oxidation conditions and at 80 °C for 168 h show. The sensitivity of oxidation to such effects may be a partial explanation as to why the reproducibility of oxidation reactions can be rather limited. Caution should be used in developing semi-quantitative or quantitative relationships from such data due to the reproducibility and repeatability; however, trends are clearly visible.

4 Conclusions

The fatty acid composition of oxidized biodiesel was evaluated using previously developed ^{1}H-NMR methods for determining the fatty acid composition of fats and oils and their methyl esters. The results show an increase in saturated and monounsaturated species. The original ω-3 double bond in 18:3 is present in larger amounts in the oxidized samples than the susceptibility of 18:3 to oxidation may indicate, showing that other double bond positions are preferentially affected in 18:3. However, it is unlikely that 18:3 is retained. The results coincide with increasing kinematic viscosity and acid values. Oxidation of biodiesel is very sensitive to the surface area of the biodiesel with air.

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